SPECIAL ARTICLES

CIRCULATION OF AMNIOTIC FLUID AND ITS MEDIATING ORGANS

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The existence of amniotic fluid was known to ancient physicians but its rapid circulation remained undiscovered until Flexner studied the disappearance of radioactive tracers injected into the amniotic cavity.[1,2] His calculations forced him to conclude that the amniotic fluid had been completely replaced within three hours. Subsequent investigations confirmed this rather rapid turnover both in man and animal.[3,4]

The mechanism by which this circulation occurs and the organs mediating it are not clearly identified. There are however vaguely understood theories, among which the most widely accepted are the following: 1. the amniotic fluid is formed by urine, excretions from the respiratory system and the refuse from the gastrointestinal tract; and 2. the fluid is absorbed by the lungs and the alimentary tract of the fetus, and from there it finally gains access to the fetal bloodstream.

These theories as well as the evidence quoted in their favor have been criticized and their validity has been doubted.[5,6] A clear understanding of the circulation of the amniotic fluid and the organs responsible for it, on the other hand, may clarify numerous problems in the physiology of gestation including fetal nutrition, electrolyte balance, and development of immunity.

Materials and Methods

Ewes in the last month of pregnancy were anesthetized with sodium pentobarbitol (25 mg Kg) and atropine sulfate (.30 mg). The trachea was intubated and under sterile conditions the abdomen was opened at the midline. A transverse incision was made in a relatively avascular area of the gravid uterus and its layers were individually dissected and incised. When the chorion was reached approximately 300 ml of the amniotic fluid was aspirated into a sterile container. Through a small incision the fetus was

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extracted from the amniotic cavity without causing embarrassment to the umbilical circulation. Its muzzle was immediately inserted into a tightly fitting rubber glove, and the fetus was placed in a plastic bag containing warm Ringer's solution. It was held immersed in this medium at the level of the maternal uterus (Figure 1). The umbilical vessels were frequently checked to insure unimpaired blood supply to the fetus.

Figure 1. Actual photograph of operative procedure. The fetus is seen at the left enclosed in white plastic bag. The umbilical cord connects the live fetus to the fetal membranes. Number 1 indicates the amniotic fluid; number 2 shows a cotyledon with vessels as seen from the amniotic cavity; number 3 shows the cord; number 4 shows the abdomen on both sides of which the forelegs of the fetus are seen; number 5 shows the glove surrounding the fetal head; and number 6 shows amniotic membrane seen from its fetal aspect.

The amniotic cavity was examined to avoid the possibility of damage to the membranes. The amniotic fluid was then replaced and 10 to 15 microcuries of radioactive sodium ($Na^{22}Cl$) were added and stirred to insure a homogeneous mixture. The arrival of the radiotracer in the maternal blood was monitored by a scintillation detector pointing to the heart. Count rates significantly above the background rate were attained four to seven minutes after addition of the radionuclide, at which time the maternal uterine vessels and umbilical cord were immediately clamped and severed. Samples were taken from the amniotic liquid and a hysterectomy was performed. Specimens of the maternal rectus abdominis muscle, the uterine wall, and blood were obtained. The operative wound was then repaired and postoperative care was given to the mother sheep.

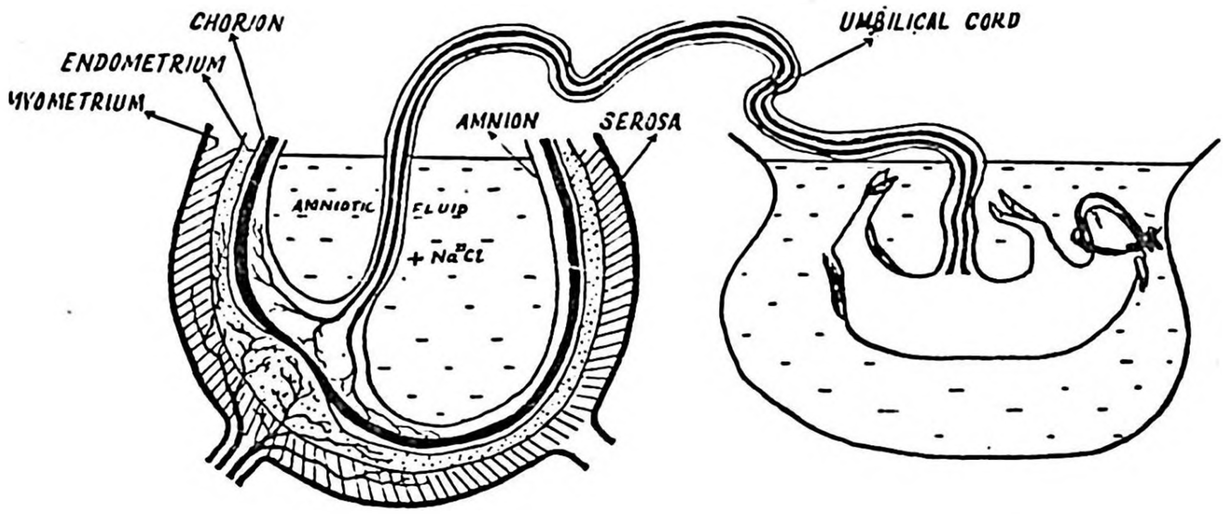

Figure 2. Schematic representation of operative procedure.

After the death of the fetus, samples of approximately one gram were taken from most of the organs. They were removed with care to avoid contamination, washed, blotted, and weighed. Each specimen was immersed in five milliliters of concentrated nitric acid and allowed to dissolve completely. The resulting homogeneous solution was then counted in a scintillation-well detector. Aliquots of blood, urine, etc., were accurately pipetted and counted using this same system. The results were corrected for background and dilution. From these figures net counts per gram or per milliliter of the samples were calculated and assumed to be proportional to the concentration of radioactive sodium in the tissues.

Results

In those samples removed five minutes after injection, fetal membranes revealed the highest concentration of radiotracer, the cotyledons were second, and the blood third. The other fetal organs, five minutes after injection, did not contain a significant amount of the tracer (Table 1). Samples removed 20 minutes after injection also showed the highest count rates in fetal ways may contribute to the transfer of sodium from the amniotic cavity to the bloodstreams of the two organisms (Tables 1 and 2).

There is ample evidence in the literature that the histological characteristics of the fetal alveoli would have to be different should their function be absorptive.[7–9] This concept supports our results. But it is possible that, under certain conditions (especially in fetal anoxia), some amniotic fluid can be absorbed from the mucous membrane of the respiratory system.

TABLE 1

Count Rates Recovered from Samples Removed after Five Minutes of Recirculation

Fetal Tissues	Case 1	Case 2	Case 3
Membranes	3300	24,993	335
Cotyledon	832	960	296
Blood	16	25	232
Umbilical cord	0	31	0
Urine	0	–	0
Stomach contents	0	22	0
Kidney	0	0	0
Lung	0	0	0
Small intestine	0	0	0
Bladder	0	0	0
Liver	0	0	0
Stomach wall	0	0	0
Heart	0	0	0
Spleen	0	0	0
Large intestine	0	0	0
Maternal Tissue			
Blood	58	0	600
Uterine wall	52	5396	90
Uterine mucosa	689	2230	0
Amniotic fluid	173,673	395,291	–

Identification of lanugo hair in the gut was claimed by several observers in the past.[6,10] From this they concluded that the fetus swallows the amniotic fluid periodically and that deglutition is responsible for circulation of the amniotic fluid. This liquid and its individual constituents could reach the fetal bloodstream after being absorbed by the intestines. According to this theory the human fetus would have to drink over a liter of fluid every hour in order to explain the large volume turnover. Jeffcoate thinks this improbable. In our earlier experiments we found that deglutition could not account for the appearance of the radionuclide in the maternal blood in less than five minutes.[11] This same conclusion is further supported by these experiments, in which the muzzle of the fetus was tightly enclosed in a rubber glove, making swallowing impossible.

When the circulation period, from injection of the radiotracer to clamping of the vessels, was extended to 20 minutes, the results were different. In this case, the concentration of radioactive sodium in the fetal bloodstream and the viscera was quite high (Table 2). The sequence from high to low concentrations of the radiotracer in the visceral organs suggests that the differences are more probably related to their blood content and partition of circulatory volume rather than to their role in mediating the transfer of amniotic fluid.

TABLE 2

Count Rates Recovered from Samples Removed after Twenty Minutes of Recirculation

Fetal Tissues	Case 4
Membranes	4175
Cotyledon	2661
Bladder	824
Kidney	817
Skin	749
Umbilical cord	660
Small intestine	641
Fetal blood	531
Liver	481
Stomach wall	470
Heart	458
Abdominus rectus muscle	335
Lung	269
Amniotic fluid	269
Spleen	252
Fetal urine	220
Brain	26
Large intestine	0
Maternal Tissue	
Uterine wall	479
Uterine mucosa	90

It is interesting that in the last case the fetal urine contained a very high concentration of radioactive sodium (Table 2). Kidney and bladder revealed more radioactivity than the other organs. These findings confirm that part of the old theory which states that the kidneys contribute to the formation of amniotic fluid.

One consistent observation in all four cases is the high concentration of the tracer recovered from maternal uterine mucosa and myometrium. This could be caused by passage of the radionuclide directly from the fetal membranes to the uterine milk and to the endometrium, as a result of osmotic disequilibrium. It is most likely that this last route of transfer is slower and plays a lesser part in circulation of the amniotic fluid at this stage of pregnancy.

Summary

In the experiments reported here, the fetus was removed from the amniotic cavity during the last month of pregnancy and placed in a plastic bag, with the umbilical circulation unimpaired. Its muzzle was inserted into a tightly fitting rubber glove and radioactive sodium ($Na^{22}Cl$) was added to the amniotic fluid remaining in the amniotic cavity. The maternal blood acquired a count rate significantly above the background level in less than five minutes. The lungs and the gastrointestinal system did not contain any more radioactive sodium than the other viscera. The fact that radionuclide was recovered from the fetal tissues indicates that the fetal membranes are responsible for circulation of the amniotic fluid.

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